Mycopathologia (2006) 161: 251–254
DOI: 10.1007/s11046-005-0248-2

Entomophthora ferdinandii (Zygomycetes: Entomophthorales) causing natural infections of *Musca domestica* (Diptera: Muscidae) in Argentina

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Received 6 October 2005; accepted in final form 1 December 2005

Abstract

The identity and activity of an entomopathogenic fungus belonging to the *Entomophthora muscae* species complex and infecting *Musca domestica* in poultry houses from La Plata, Argentina, is reported. *Entomophthora* caused natural infections between September 2001 and September 2003. Primary conidia of this fungus were on average $29.5 \pm 1.2 \times 23.4 \pm 2.4$ μm and contained, on average, 10.5 ± 0.1 nuclei (range: 7–15) with an average diameter of 4.8 ± 0.1 μm. This fungus is identified as *E. ferdinandii* Keller (this specific epithet includes a nomenclaturally required spelling correction); this is a first record of *E. ferdinandii* in South America and of any member of the *E. muscae* species complex from flies in Argentina.

Key words: entomopathogenic fungi, *Entomophthora muscae* species complex, house flies, new geographical record, poultry house

Introduction

Entomopathogenic fungi cause epizootics in house flies under natural conditions. The *Entomophthora muscae* (Cohn) Fresenius species complex (Entomophthorales: Entomophthoraceae) frequently causes epizootics and is an important mortality factor for several species of Diptera belonging to the families Muscidae, Calliphoridae, Anthomyidae, Sarcophagidae, Drosophylidae and Syrphidae. Its presence has been reported from the USA [1] and from Europe [2–4]. In South America the *E. muscae* species complex has only been reported previously from Chile [5] and Brazil [6].

Entomophthora muscae is considered to be a species complex [7–10]. The resolution of this species complex and the later elaboration of the overall taxonomy for the genus *Entomophthora* have been based mainly on comparisons of the dimensions of primary and secondary conidial sizes, on the number and size of nuclei in the primary conidia, and, to a lesser extent, on the host(s) affected [9–12]. The presence of the *E. muscae* species complex in the Neotropics is at present poorly documented, and it has not been reported previously from Argentina. The objective of this present study was to determine wheter *E. muscae* group of fungi were present in a natural house fly population repeatedly

sampled in La Plata, Argentina and to identify any such fungi found.

Materials and methods

Study site

A caged layer poultry house ranch in western La Plata county at "El Peligro" (interstate Highway No. 2, 34°05′05″ S, 58°10′26″ W), Buenos Aires State, Argentina, was monitored weekly over a period of 24 months from September 2001 through September 2003 to assess the presence of *Entomophthora* sp. in house flies.

Adult house flies were collected with a sweep net placed in a plastic screened cage of 30×30×30 cm (Megaview®) and carried to the laboratory. House flies were fed on a mix of 1:1 distilled water + milk powder (3% fat content). Dead flies were removed daily for up to 7 days after field collections and they were observed with a stereo microscope for signs of *Entomophthora* infection. Infected cadavers were preserved in 70% ethanol for further identification and for future uses in molecular studies of the fungal samples.

Fungal identification

Fungal identification was based on the characters of species from the genus *Entomophthora* presented by Keller [9–12]. Primary conidia were collected by placing a freshly killed house fly between coverslips in a humid chamber. For each fungus-killed fly the lengths and widths of primary and secondary conidia (a total of 20 conidia from each individual) were measured from fresh preparations in lactophenol/aceto-orcein [10] observed microscopically with phase contrast at 400× magnification with an Olympus CH 30 microscope. The number of nuclei in primary conidia was quantified, and nuclear diameters were measured. For secondary conidial discharge, a glass slide was placed above a slide with primary conidia for 4 h, and secondary conidia then were mounted in lactophenol-cotton blue 0.01%. The fungus identification was based on mean number of nuclei in primary conidia and diameter of conidia according to the classification of Keller [11]. Hyphal bodies were observed by taking hemolymph samples from infected flies with a 100 μl Micro-Pipets® (Fisher). Attempts were made to isolate the fly fungus in axenic culture using hyphal bodies as inoculum in both Grace's insect tissue culture medium (Gibco BRL) and GLEN [13]. Both media were supplemented with 10% fetal bovine serum (FBS). None of these attempts resulted in successful isolations.

In vivo *culture of* Entomophthora

In vivo culture of the Argentinean *Entomophthora* was started in September 2001 from dead infected flies captured in the poultry house. Healthy flies were kept in cages of (30×30×30 cm, plastic screened) (Megaview®), fed with a mix of milk powder (3% fat content) and distilled water (1:1) and kept at 22 ± 1 °C. Flies infected with *Entomophthora* were placed over the cages together with healthy house flies following the method of Kramer and Steinkraus [14]. The purpose of the *in vivo* culture was to obtain sufficient number of freshly sporulating cadavers for morphological studies purposes.

Results and discussion

Fungal taxonomy

The examination of the material demonstrated that this fungus from Argentinean adults of *Musca domestica* (Diptera: Muscidae) belonged to the *Entomophthora muscae* species complex. The campanulate and apiculate primary conidia of this fungus were 29.5 ± 1.2×23.4 ± 2.4 μm (range: 19.6–35.5×18.9–30.8 μm) (pooled data from measurements from six infected flies) (Figure 1). Primary conidia included 10.5 ± 0.1 nuclei (range: 7–15), (N = 186; pooled measurements from 11 infected flies corresponding to different samplings) with an average diameter of 4.8 ± 0.1 μm (range: 4.7–5.5 μm). Secondary conidia measured 16.6 ± 1.3×12.8 ± 1.2 μm (range: 11.8–16.0×9.5–14.2 μm) (N = 40). Data showed are means ± SE. The conidiophores of this fungus were unbranched, and its hyphal bodies in the host hemocoele were spherical to slightly ellipsoidal. Hyphal bodies were not measured because scarse material was available. Dead infected flies were attached to substrate by blunt-ended hyphae (rhizoids) radiating from the proboscis. Neither cystidia nor resting spores were found.

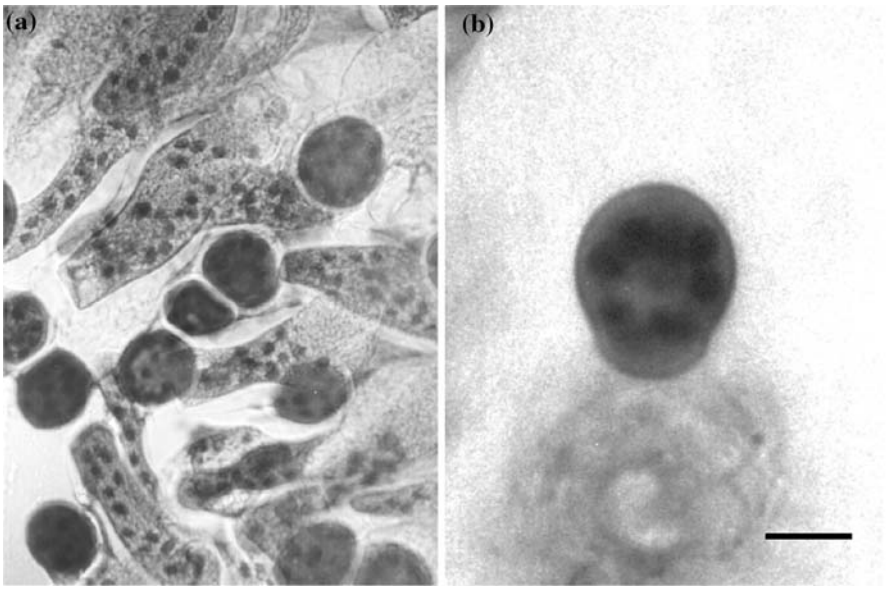

Figure 1. Entomophthora ferdinandii. (a) Conidiophores and primary conidia sampled from the external hymenial layer on an infected fly. (b) Multinucleate primary conidia. Scale bar: (a): 20 μm, *(b): 10* μm.

As treated by Keller [9–11], *Entomophthora muscae* in the nomenclaturally broad sense includes *E. schizophorae* Keller and Wilding in Keller with 4–8 nuclei per conidium, *E. scatophagae* Giard with 15–18 nuclei, *E muscae* with 15–20 nuclei, and *E. syrphi* Giard with 19–22 nuclei. A recent study on the molecular systematics of the genus *Entomophthora* has supported the validity of *E. schizophorae* and *E. syrphi* as species distinct from *E. muscae* [3].

E. muscae s. str. (in the nomenclaturally strict sense) [12] produces campanulate/apiculate primary conidia of 26.9–31.1×20.4–24.2 μm (range: 21–35×16–29 μm) with 15.2–20.2 nuclei (range: 10–27) having a diameter of (3.5–) 3.9–4.4 (–5.5) μm; secondary conidia are 19.3–24.2×15.1–19.1 μm (range: 16–28×12–23 μm). Hyphal bodies subspherical, subellipsoidal, subovoid, rarely spherical. The conidiophores, (18–) 23.2–30.2 (–39) μm in length, are unbranched and apically swollen. Resting spores and cystidia of *E. muscae* s.l. are not known to occur naturally in the original host for *E. muscae*, *M. domestica* (11), but are found to occur in natural hosts from Anthomyiidae [15]. In laboratory experiments, resting spores can be induced in *M. domestica* [16].

Keller (1984) characterized one variant form within the *E. muscae* species complex as 'type B', with primary conidia 22.7–26.6×17.9–22.7 μm (range: 21–30×16–27 μm) containing 9.8–11.2 nuclei (range: 6–16) having a diameter of 3.9–4.0 μm (range: 3.5–5.0 μm), and secondary conidia 17.7–17.8 μm long (range: 16–19 μm). The conidiophores of *E. muscae* 'type B' were characterized as being unbranched and containing 10.1–10.3 nuclei (range: 7–14). More recently, Keller described this fungus as *E. ferdinandi* Keller [11], and additionally noted that the hyphal bodies were subspherical to slightly ellipsoidal. Rules in the International Code of Botanical Nomenclature [17] for latinizing personal names as specific epithets (Article 60.11 and Recommendation 60C.1) require the correction of this species name to *E. ferdinandii*.

Entomophthora was previously reported to affect *M. domestica* in South America as *E. muscae* s.l. from Chile [5] and Brazil [6]. The only measurements presented by Aruta et al. [5] and Madeira [6] were those of primary conidia: 30.6×25.3 and 23.4×19.9 μm and with 8–19 nuclei, respectively. The Brazilian fungus reported as *E. muscae* [6] has primary conidia with more nuclei (average: 12.5) and primary conidia are smaller (average: $23.4 \pm 4.7 \times 19.9 \pm 9.9$ μm) than the Argentinean fungus reported here. It might also be referable to *E. ferdinandii*, but such a re-identification cannot be done without more detailed morphological information about the Brazilian fungus. While some primary conidia of the

Argentinean fly fungus are larger than those reported for *E. ferdinandii* [11], the number of conidial nuclei and also the size of its secondary conidia are well within those described for *E. ferdinandii*. The slightly larger size ranges for both the primary conidia and their nuclei of the Argentinean fungus than were described for *E. ferdinandii* [11] could easily be due to differences in preparative techniques as documented by Humber [18]. Nonetheless, there is little doubt that the Argentinean and possibly the Brazilian fly fungi are better identified as *E. ferdinandii* than as *E. muscae* s. str. [11, 12]. It is noteworthy that *E. ferdinandii* caused high infection in the flies. The highest natural infection percentages were observed during the spring of 2001 (50.6%) and the autumn of 2002 and 2003 (33.2 and 68.4%, respectively).

The identification of the fungus reported here appears to be fully consistent with *E. ferdinandii* except for the family of flies affected. To date, *E. ferdinandii* is known only from the *Anthomyiidae* whereas the Argentinean fungus is from the *Muscidae*. The infectivity of the Argentinean fungus for anthomyiid flies has not yet been tested, but several *Entomophthora* species pathogenic for Diptera can attack hosts from more than one family [11], and the difference in host family observed here does not seem likely to be significant. That neither resting spores nor cystidia were observed in our material is consistent with the general rarity of resting spores and absence of cystidia from *Entomophthora* species [11, 12]. This is the first record of *E. ferdinandii* in house flies for South America and Argentina, and is the first report of this species from outside Europe.

Acknowledgments

We thank the National Research Council of Argentina for partial financial support of this research, and to S. Keller comments about fungal material measurements of an earlier version of this manuscript.

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